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Abundance in Bottom Sediments and Hatching Requirements of Eggs of *Centropages hamatus* (Copepoda: Calanoida) From the Alligator Harbor Region, Florida

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Abstract. The distribution and abundance of *Centropages hamatus* eggs in the upper 5 cm of the sea-bottom of the Alligator Harbor region, Florida, was determined. The hatching of these eggs at 4, 10, 15, 19, and room temperature (20–25°C) was monitored in the laboratory. Hatching only occurred at temperatures less than 20°C. Eggs produced by *C. hamatus* individuals that were reared in the laboratory at 19°C, 20L–4D did not hatch at 19°C. A 60% hatch was induced by incubating these eggs at room temperature (22–25°C) for 60 days followed by cooling at 15°C. The results indicate that *C. hamatus* produces diapause eggs that are capable of surviving warm temperatures characteristic of late spring and summer months. Since planktonic phases of this species are not present in the water column at temperatures greater than 20°C, it appears that diapause is a critical life history stage without which the life cycle would be broken.

Introduction

In temperate-boreal waters many planktonic copepod species undergo marked fluctuations in seasonal abundance to the extent that some species disappear entirely from the water column for portions of the year. The renewed development of benthic diapause eggs plays a fundamental role in the perpetuation of such species year after year in these regions (as reviewed by Grice and Marcus, 1981; Uye, 1985). However, the widespread geographic significance of diapause eggs is not clear since all studies have focused on temperate systems. Studies of copepod population dynamics in some sub-tropical and tropical waters have led investigators to suggest that resting eggs occur in these areas as well (Tranter and Abraham, 1971; Reeve, 1975; Fleminger, 1979), but the actual existence of such eggs has not been demonstrated. Moreover, it is not clear if such eggs would be subitaneous eggs whose development had been delayed temporarily due to unfavorable conditions in the sediment (*e.g.*, low oxygen) or diapause eggs. Unlike subitaneous eggs, which are capable of developing without any delay, diapause eggs must first complete a refractory phase during which time they will not hatch even if conditions are suitable. Hatching can only occur once this phase is completed and then only if conditions (*e.g.*, temperature, salinity, oxygen) are suitable. The subitaneous and diapause eggs of some copepod species can be clearly distinguished on the basis of external morphology (*e.g.*, the presence or absence of spines), but others are not morphologically distinct and can only be classified by determining the conditions that are necessary for hatching to occur (as reviewed by Grice and Marcus, 1981).

A preliminary analysis of bottom sediments from the Alligator Harbor region, Florida, in the late spring and summer 1988 (Marcus, unpub.) revealed the existence of benthic resting eggs of several planktonic copepod species: *Labidocera aestiva, L. scotti, Acartia tonsa, Centropages furcatus,* and *C. hamatus.* Species identifications were made by comparing the morphology of eggs (*e.g.*, diameter, surface ornamentation, color) isolated from the sediments to the morphology of eggs produced by females that were maintained in the laboratory, and by rearing nauplii that hatched from the eggs to late-copepodite and adult stages which could be unequivocally identified. Since adults of *C. hamatus* were not present

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in the water column at the time of the sediment collections, the species was chosen for further study, because the absence of planktonic stages in this northern subtropical embayment suggested that the eggs that were found were diapause eggs. These eggs were spherical, approximately 72 μm in diameter, and covered with spines that were approximately 18 μm in length.

Centropages hamatus (Lilljeborg) is a geographically widespread species. Along the coast of North America it has been reported from the Straits of Belle Isle (Pinhey, 1926) to Alligator Harbor in the Gulf of Mexico (Grice, 1956). In Alligator Harbor, the species has been described as a characteristic member of the winter copepod population occurring in the water column from November to April (Grice, 1956). In areas along the Atlantic coast of the United States south of Cape Cod, Massachusetts, the species has been reported to occur seasonally, typically during the winter and spring (see Deevey, 1960). Occasionally, however, when summer temperatures were abnormally low in these areas, it occurred throughout the year, *e.g.*, in Delaware Bay (Deevey, 1960). In the Gulf of Maine (Bigelow, 1926) and on Georges Bank (Davis, 1987), the species has been reported to occur during the late summer and fall.

C. hamatus has been reported to produce two morphological types of eggs in the White Sea (Pertzova, 1974) and it was later suggested (Grice and Marcus, 1981) that the eggs produced in the fall were diapause eggs. Other evidence for the existence of resting eggs was the appearance of *C. hamatus* nauplii following the laboratory-incubation of bottom sediments that were collected from Buzzards Bay, Massachusetts, Georges Bank, the English Channel, and the southern North Sea (Marcus, 1984, unpubl.; Lindley, 1986). However, it is not clear whether the nauplii that appeared were derived from diapause eggs or subitaneous eggs that were delayed in development due to adverse conditions in the sediments.

This study was conducted to determine the distribution and abundance of eggs of *C. hamatus* in the bottom sediments of the Alligator Harbor region. The hatching requirements of these eggs, and eggs obtained from animals that were reared in the laboratory, were ascertained. The results show that *C. hamatus* does produce diapause eggs. It is suggested that these eggs enable the species to survive warm summer temperatures.

Materials and Methods

Determination of egg distribution in the sediments

Sampling was conducted from July 9, 1988, through October 20, 1988, at six stations in the Alligator Harbor region, Florida (Fig. 1). At each station sediments were collected with a hand-held pole corer (Lively, unpubl.),

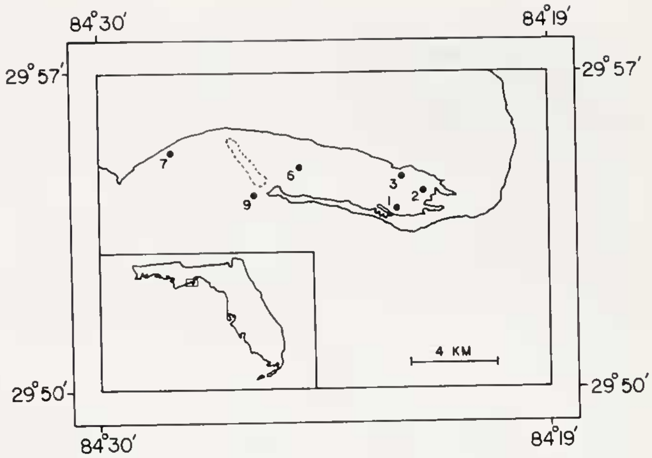

Figure 1. Location of six sampling stations in the Alligator Harbor region, Florida.

water depth was recorded from a meter scale on the pole, and surface temperature and salinity were determined with a Y.S.I. Model 33 S-C-T Meter. With the pole corer, cylindrical core tubes (4.8 × 30 cm D × L) were slowly pushed into the sea-bottom to a depth of about 15 cm. A ball valve was then triggered to close, trapping the sediment, and the unit was hauled to the surface. Cores were kept in an insulated container from the time of collection until they were returned to the laboratory. For some collections, the cores were additionally packed in ice. In the laboratory, cores were divided at intervals as fine as 2 mm and as coarse as 1 cm using a core extruder (Fuller and Butman, 1988). The material in each layer was suspended in 5 μm filtered seawater, sonicated for 30 s (#4 setting, Branson Sonifier Cell Disruptor 200), filtered through a 48 μm Nitex screen, suspended in a concentrated solution of table sugar (1:1 sugar:distilled water), and centrifuged for 3 min. The material remaining in suspension was filtered through a 48 μm screen, washed thoroughly with seawater, and transferred to a dish containing seawater. The final fraction was examined under a dissecting microscope and eggs of *C. hamatus* were counted and transferred to small wells containing seawater. The eggs were kept at room temperature (22–25°C) and monitored for hatching.

Qualitative plankton samples were also obtained from surface waters throughout the study. A 153 μm mesh net was towed for 2 to 5 min and the collected material was returned to the laboratory in insulated containers. Samples were examined under a dissecting microscope to determine if *C. hamatus* adults were present.

Determination of hatching requirements

Experiment 1 was conducted with eggs collected on July 9. The sediment samples were held at 4 ± 1°C for 2

Table I

Mean number (×10⁴) and standard deviation (S.D.) of eggs m⁻² of Centropages hamatus *in the top 5 cm of bottom sediments from the Alligator Harbor region, Florida, at each station for the period from July 9 through October 20, 1988*

Station	1	2	3	6	7	9
Water depth (m)	1.6	1.6	1.6	2.1	3.5	4.0
# of cores	3	2	2	2	10	3
Mean	1.8	0.4	0.0	0.7	7.3	3.6
S.D.	0.9	0.2	0.0	0.5	6.2	2.1

to 4 days prior to isolation of the eggs. The eggs were then held at room temperature (22–25°C) for 5 to 7 days. During this time no eggs hatched and the eggs were subsequently distributed into five jars (10 eggs/jar). Two jars were placed at 4°C, and three were held at room temperature (22–25°C). After 59 days the jars at 22–25°C were transferred to 10, 15, and 19 ± 1°C since none of the eggs had hatched.

Experiment 2 was conducted with a mixture of eggs from two collection dates, July 9 and July 26. In this case the sediment samples were held at 4°C for as much as 11 days prior to isolation of the eggs. The initial incubation period at room temperature (22–25°C) ranged from 1 to 11 days. As in Experiment 1, no eggs hatched during the warm period so 119 eggs were placed in a jar and stored at 4°C.

Experiments 3 and 4 were conducted with eggs collected on September 14 and October 20, respectively. Eggs were not exposed to an initial period at 4°C prior to their isolation, nor a pre-incubation at room temperature. Rather, as soon as the eggs were isolated from the samples they were distributed into jars and placed at either 4, 10, 15, 19, or room temperature (20–24°C). In Experiment 3 each jar contained 15 eggs, but there were not enough eggs to have replicates at each temperature. In Experiment 4 each temperature condition was tested in duplicate with 10 eggs/jar.

Hatching was monitored periodically. Some of the nauplii that hatched were reared to reproductive maturity on a mixed diet of 4 dinoflagellates (*Gymnodinium nelsoni*, *Gonyaulax polyedra*, *Prorocentrum micans*, and *Scrippsiella trochoidea*) at 19°C and 20L–4D (h light–h dark). The eggs produced by these animals were kept in a dish of seawater at 19°C for 3 days. None of the eggs hatched so they were distributed into jars (15 eggs/jar) which were then held at room temperature (22–25°C) for periods ranging from 2 to 60 days. Each jar was transferred to 15°C following its particular incubation interval at room temperature. Hatching at the cooler temperature was monitored daily.

Results

Large numbers of eggs of *C. hamatus* were found in the sediments of the Alligator Harbor region at water depths ranging from 1.6 to 4.0 m (Table I). The eggs occurred as deep as 5 cm (the maximum depth analyzed in this study). The average abundance of eggs in the top 5 cm ranged from 0 to 7.3×10^4 eggs m^{-2} (Range: 0 to 2.1×10^5). Greater densities of eggs were found in the deeper waters outside of Alligator Harbor at Stations 7 and 9. Eggs were found from July through October and during this time adults of *C. hamatus* were not present in the water column. During July and August, surface water temperature and salinity ranged from 26.0 to 28.5°C and 32 to 35‰, respectively. By October, water temperature and salinity had declined to 18.0 to 20.0°C, and 27 to 31‰, respectively.

Eggs collected in July (Experiments 1 and 2) did not hatch at 22–25°C even after 2 months of incubation. Hatching occured at each of the other incubation temperatures, and the time to hatching was inversely related to temperature (Fig. 2). Eggs collected in September and October (Experiments 3 and 4) hatched at each of the incubation conditions. The cumulative hatch for the eggs held at 4, 10, 15, and 19°C are shown in Figure 2. As in Experiments 1 and 2 hatching was most rapid at the warmer temperatures. In Experiments 3 and 4 the cumulative hatch of eggs held at room temperature (20–24°C) was 33% and 90%, respectively. In these experiments hatching occurred in the jars at room temperature only after the temperature had dropped to 20°C.

Eggs that were produced by the laboratory-reared animals hatched at 15°C, but only after prolonged exposure to warm room temperatures (22–25°C). The first hatch (*i.e.*, one nauplius) occurred after 12 days at 15°C, in the

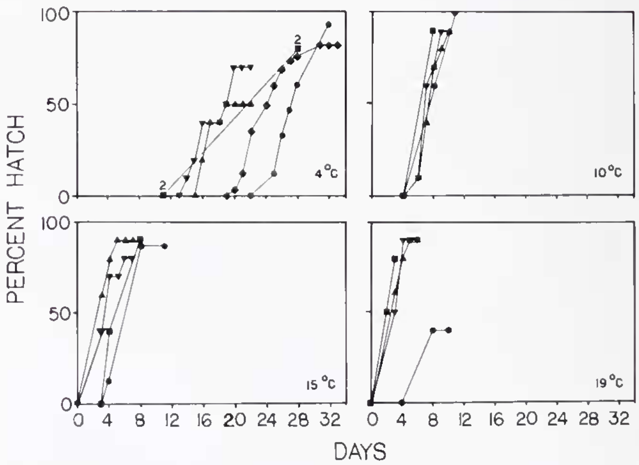

Figure 2. Cumulative percent hatch of *Centropages hamatus* eggs collected from bottom sediments and incubated in the laboratory. Experiments 1 (■), 2 (♦), 3 (●), and 4 (▲,▼) duplicates).

jar kept at room temperature for 38 days. A greater hatch (60%) occurred in the jar held at room temperature for 60 days. Hatching began after four days at 15°C and continued for 44 days.

Discussion

The abundance of eggs of *C. hamatus* in the sediments of the Alligator Harbor region, Florida was high. The greatest density, 2.1×10^5 m^{-2}, is of the same order of magnitude as that reported for eggs of *Labidocera aestiva* in Buzzards Bay, Massachusetts (Marcus and Fuller, in press). Higher numbers tended to occur in deeper water outside the embayment. The fact that these eggs hatched upon incubation in the laboratory indicates that eggs of *C. hamatus* can survive for as long as 5 months in bottom sediments. This is a minimum estimate and assumes that the eggs collected in October were produced in April just prior to the disappearance of the species from the water column. In the Alligator Harbor region, adults of *C. hamatus* occur from November through April (Grice, 1956). During the time that *C. hamatus* is absent from the water column temperatures typically range from approximately 20 to 32°C (Grice, 1956). Since the results of this study indicate that eggs only hatch at temperatures equal to or less than 20°C, one might conclude that eggs are simply inhibited from hatching during the late-spring and summer periods due to warm temperatures. When the water temperature drops below 20°C in October, the eggs hatch and give rise to the adults that appear in November.

The hatching response of eggs that were produced by animals reared at 19°C, 20L–4D indicates that *C. hamatus* has the potential to produce diapause eggs (according to the criteria of Grice and Marcus, 1981). None of the eggs produced by laboratory-reared animals hatched at 19°C initially, but some of these eggs did hatch at 15°C after prolonged incubation at warm room temperatures (22–25°C). Although, 20L–4D constitutes an abnormally long daylength never experienced by *C. hamatus*, the results demonstrate that the species has the biological capacity to produce diapause eggs. In a previous study (Marcus, unpubl.), *C. hamatus* from Woods Hole, Massachusetts, were reared at 19°C, 12L–12D and produced thousands of eggs. Many of these eggs hatched within a few days at 19°C, but many did not hatch. The precise percentage of eggs that hatched was not determined. Some of the unhatched eggs were placed in jars containing filtered seawater, stored at room temperature for approximately 3 months, and then transferred to 5°C. No hatch occurred while the eggs were held at room temperature, but after the eggs were transferred to 5°C some hatch occurred after 13 days with large numbers of nauplii noted after 17 days. Although a quantitative record was not kept, the results are also indicative of diapause. Since the eggs produced by Woods Hole animals were kept for 3 months at room temperature it may be that the eggs of the laboratory-reared *C. hamatus* from Florida were not incubated sufficiently long to yield a rapid and synchronous hatch at cool temperatures (*i.e.*, 15°C). Unlike the report of Pertzova (1974) on *C. hamatus* from the White Sea, only one morphological type of egg was observed for *C. hamatus* from Woods Hole and Florida. It was spherical and uniformly covered with spines.

Only two other studies have reported on the hatching requirements of resting eggs produced by winter-spring marine copepods. Sullivan and McManus (1986) showed that some eggs produced by *Acartia hudsonica* from Narragansett Bay, Rhode Island only hatched at cold temperatures *i.e.*, 5°C. A requirement for an intervening period of warm temperatures was not demonstrated. However, the eggs of *A. clausi* from Japanese waters appear to require a period of warm temperatures followed by chilling to induce hatching (Uye, 1985).

Marcus (1979) showed that the refractory phase (*i.e.*, the period of time during which diapause eggs will not hatch even if environmental conditions are favorable) of eggs of *L. aestiva* was shortened by chilling at 5°C, but that diapause eggs would eventually hatch after several months at warm temperatures even if they had not been chilled. The difference was that pre-chilled eggs hatched synchronously. It is not clear if the opposite condition holds for *C. hamatus; i.e.*, would eggs hatch at temperatures less than about 20°C if they were not exposed to a period of warm temperatures? The temperature conditions that are required for embryonic development and hatching must be taken into account to predict the contribution of benthic diapause eggs to the population growth of species. For example, along the northeast coast of the United States it is likely that diapause eggs residing on the sea-bottom rarely experience temperatures above 20°C. If exposure to temperatures warmer than 20°C is required for hatching, then these eggs should not be considered as a source of potential recruits to the planktonic population. On the other hand, if such warm temperatures are not necessary, then such eggs represent an important potential source of nauplii for growth of the planktonic population. Since the annual environmental cycles in waters of the western north Atlantic are different than the conditions in the northeastern Gulf of Mexico, it is likely that the precise conditions that are necessary for the induction, maintainence, and termination of diapause are different. Regardless of these geographic differences, in areas where *C. hamatus* disappears entirely from the water column for portions of the year, diapause eggs enable survival of individuals during warm seasons and must be essential for the perpetuation of species year after year.

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Literature Cited

Bigelow, H. 1926. Plankton of the offshore waters of the Gulf of Maine. *Bull. U. S. Bur. Fish.* **40:** 1–509.

Davis, C. 1987. Zooplankton life cycles. Pp. 256–267 in *Georges Bank*, R. Backus and D. Bourne, eds. MIT Press, Cambridge, Massachusetts.

Deevey, G. 1960. The zooplankton of the surface waters of the Delaware Bay region. *Bull. Bingham Oceanogr. Coll.* **17:** 5–53.

Fleminger, A. 1979. *Labidocera* (Copepoda:Calanoida): new and poorly known Caribbean species with a key to species in the western Atlantic. *Bull. Mar. Sci.* **29:** 170–190.

Fuller, C., and C. A. Butman. 1988. A simple technique for fine-scale vertical sectioning of fresh sediment cores. *J. Sed Petrol.* **58:** 763–768.

Grice, G. 1956. A qualitative and quantitative seasonal study of the Copepoda of Alligator Harbor. Florida State Univ. Stud. No. 22. Papers from the Oceanogr. Inst. No. **2:** 37–76.

Grice, G., and N. Marcus. 1981. Dormant eggs of marine copepods. *Oceanogr Mar. Biol. Ann. Rev.* **19:** 125–140.

Lindley, J. 1986. Dormant eggs of calanoid copepods in sea-bed sediments of the English Channel and southern North Sea. *J. Plank. Res.* **8:** 399–400.

Marcus, N. 1979. On the population biology and nature of diapause of *Labidocera aestiva* (Copepoda: Calanoida). *Biol. Bull.* **157:** 297–305.

Marcus, N. 1984. Recruitment of copepod nauplii into the plankton: importance of diapause eggs and benthic processes. *Mar. Ecol. Prog. Ser.* **15:** 47–54.

Marcus, N., and C. Fuller. In press. Distribution and abundance of eggs of *Labidocera aestiva* (Copepoda:Calanoida) in the bottom sediments of Buzzards Bay, Massachusetts, U.S.A. *Mar. Biol.*

Pertzova, N. 1974. Life cycle and ecology of a thermophilus copepod, *Centropages hamatus* in the White Sea. *Zool. Z.* **53:** 1013–1022.

Pinhey, K. 1926. Entomostraca of the Belle Isle Strait expedition, 1923, with notes on other planktonic species. Part I. *Contr. Can. Biol., N.S* **3:** 1–55.

Reeve, M. 1975. The ecological significance of the zooplankton in shallow sub-tropical waters of south Florida. Pp. 352–371 in *Estuarine Research*, Vol. 1, L. Cronin, ed. Academic Press, New York.

Sullivan, B., and L. McManus. 1986. Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A.* tonsa in Narragansett Bay, Rhode Island: temperature and resting egg production. *Mar. Ecol. Prog. Ser.* **28:** 121–128.

Tranter, D., and S. Abraham. 1971. Coexistence of species of Acartiidae (Copepoda) in the Cochin Backwater, a monsoonal estuarine lagoon. *Mar. Biol.* **11:** 222–241.

Uye, S. 1985. Resting egg production as a life history strategy of marine planktonic copepods. *Bull. Mar. Sci.* **37:** 440–449.